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# An implanted reservoir of morphine solution for rapid induction of physical dependence in rats

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**Summary**

1. Rats were dosed continuously with morphine hydrochloride by giving a daily dose through tubes connected to small, subcutaneously implanted reservoirs. Morphine was withdrawn by washing out the reservoir with drug vehicle. The daily dose of morphine, or substitute drug received by each rat was determined by difference by estimating the drug remaining in reservoir washings.

2. Withdrawal symptoms were more pronounced after 9 days than after 4 days of dosing with morphine.

3. Body weight loss, maximal at 24 h, and increased defaecation during the first 7 h were the chief physiological signs of morphine withdrawal. The body weight loss was the result of hypodipsia and anorexia exacerbated by increased defaecation.

4. When substituted for morphine in the reservoir, methadone and codeine completely prevented body weight loss and increased defaecation, while pethidine was effective against increased defaecation, but not against 24 h body weight loss. The opiate-antagonist analgesics pentazocine, nalorphine and cyclazocine either had no effect on withdrawal symptoms or increased their severity.

5. In morphine dependent rats under continued morphine administration subcutaneous doses of the opiate-antagonists nalorphine, cyclazocine and naloxone all precipitated the withdrawal symptoms of body weight loss and increased defaecation. The weak antagonist pentazocine caused a significantly increased defaecation, but no significant change in body weight, while the opiates pethidine, codeine and methadone had no significant effect on body weight or defaecation.

6. The advantages of inducing dependence by this method of dosing are discussed.

## Introduction

At an early stage in the development of a new analgesic drug there is a need to assess its capacity for causing physical dependence. The ability of such a drug to prevent withdrawal symptoms in morphine dependent animals is considered a useful criterion for assessing dependence liability (Seevers & Deneau, 1963 ; Halbach & Eddy, 1963).

To obtain dependent animals it is usual to administer doses of morphine for periods of 3 weeks or more (Deneau & Seevers, 1964). Furthermore, the parenteral dose frequency needs to be high (3 or 4 times per day) in order to prevent withdrawal symptoms occurring between doses (Buckett, 1964). For optimal development of dependence continuous 'neuronal exposure' to the drug is necessary (Seevers & Deneau, 1963). A simple method of dosing, which affords continuous exposure to drug, uses a pellet of morphine base subcutaneously implanted in mice (Maggiolo & Huidobro, 1961 ; Way, Loh & Shen, 1969). The disadvantages of this method are that after implantation the daily dose of morphine received from absorption of the pellet cannot be controlled and it is necessary to remove the pellet surgically, or to administer an opiate antagonist to produce withdrawal symptoms.

The method described in the present work was devised to overcome these disadvantages.

## Methods

A reservoir of morphine hydrochloride, implanted subcutaneously in rats, was used to provide a continuous dose from a single daily injection.

### *Reservoir*

Each reservoir (Fig. 1) was made of silicone rubber tubing and sheeting (Dow-Corning Silastic—Down Bros. and Mayer and Phelps Ltd.) with a cellophane membrane at one end to allow drug to diffuse out slowly. Before implantation the internal volume of each reservoir was determined and the reservoirs were then autoclaved. (A typical sample of forty-eight reservoirs had an internal volume of 0·409 ml $\pm$ 0·003 S.E.M.).

### *Implantation*

Female Charles River rats in the body weight range 180–290 g were prepared by shaving a small area of dorsal skin immediately behind the ears. Each rat was anaesthetized with halothane and a 1 cm median dorsal longitudinal incision was

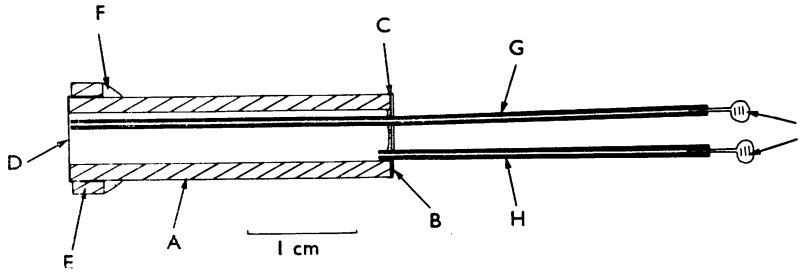


FIG. 1. Sectional diagram of the reservoir. The body of the reservoir A is a 3 cm length of Silastic tubing (I.D. 4·8 mm, O.D. 7·9 mm), which is closed at one end by Silastic sheeting B (0·5 mm thick) and sealed with Silastic cement C. At the other end a cellophane membrane D (one layer of cellophane from Visking dialysis tubing, Scientific Instrument Centre Ltd.) is held in place by a 3 mm length of Silastic tubing E (I.D. 2·8 mm, O.D. 6·0 mm) expanded over the body of the reservoir and also sealed with Silastic cement F. The reservoir is filled by means of Silastic tubes G and H (I.D. 1·0 mm, O.D. 2·2 mm), drug solutions being injected through tube G to prevent air pockets. These tubes are closed by polythene stoppers I.

made in the shaved skin. The skin was separated from the body wall for approximately 4 cm posterior to the incision and the reservoir was inserted with the cellophane membrane pointing posteriorly. The filling tubes were brought to the exterior through skin puncture wounds slightly posterior and lateral to the incision which was then sutured. Throughout the ensuing experiment rats were kept in individual cages.

### *Dosing—induction of physical dependence*

Dosing with morphine began 3 days after implantation by washing the reservoir through with 2 ml of morphine hydrochloride solution (30 mg/ml) at 09.00 h and by repeating this dose on subsequent days at the same time. Figure 2 shows the passage of morphine from the reservoir over a period of 24 hours. At no time did rats show withdrawal symptoms when dosed in this way.

### *Drugs and vehicles*

For administration by means of the reservoir, all drugs except pethidine hydrochloride were dissolved in 0·1 M citrate/phosphate buffer (pH 5·5) which prevented precipitation of morphine base. The buffer contained 0·001% benzalkonium chloride as a bacteriostat. Pethidine hydrochloride was dissolved in physiological saline. All drugs administered by subcutaneous injection were dissolved in physiological saline (10 ml/kg).

The drugs used were morphine hydrochloride, codeine phosphate, pethidine hydrochloride, methadone hydrochloride (Macfarlan Smith Ltd.), nalorphine hydro-

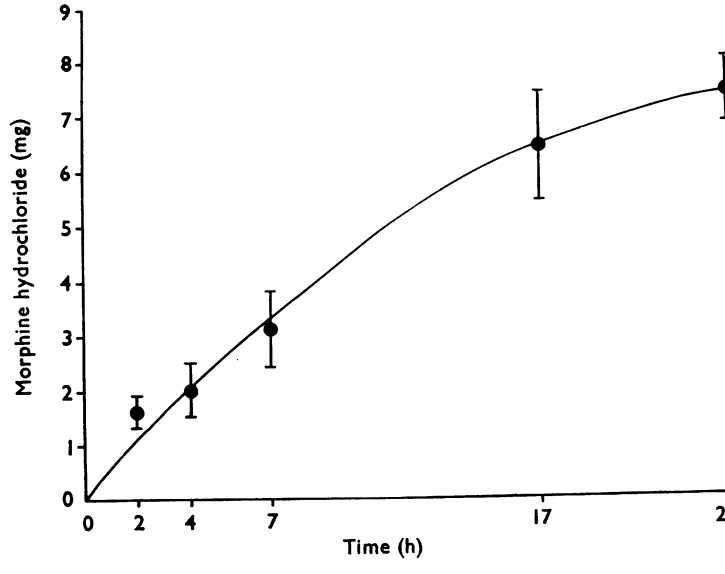


FIG. 2. Passage of morphine hydrochloride from the reservoir during the 24 h after filling with a 30 mg/ml solution. Each point represents the mean cumulative amount passing from the reservoir ($N=12$) and the vertical bars show the standard error of the mean (S.E.M.). The initial amount of morphine hydrochloride in the reservoir was 12·2 mg ± 0·3 S.E.M. The rate of diffusion of morphine hydrochloride from the reservoir was such that each 200 g rat received approximately (3 mg/kg)/h during the first hour, approximately (0·5 mg/kg)/h during the twenty-fourth hour and approximately (37 mg/kg)/day.

bromide (Wellcome) and naloxone hydrochloride (Endo). Pentazocine and cyclazocine (Bayer) bases were dissolved in molar equivalents of hydrochloric acid.

### *Dose estimation*

As the internal volume of each reservoir and concentration of drugs were known the daily dose of drug received by each rat could be determined by washing out the reservoir with vehicle and estimating the remaining drug in the washings. Morphine, codeine, pentazocine, nalorphine and cyclazocine were determined by diluting the washings with 0·1 N hydrochloric acid and estimating the drugs fluorometrically. Pethidine and methadone were estimated by converting to base with saturated sodium carbonate solution, extracting with ether and determining on an Aerograph 1520A gas chromatograph.

## Results

### *Morphine withdrawal*

Two preliminary experiments were made to investigate the effects of morphine withdrawal after 4 or 9 days of exposure to the drug. Unless otherwise stated groups of six rats were used. In each experiment three groups of rats received drugs by means of the reservoir. One group received buffer and acted as a vehicle dosed control. Two groups received morphine hydrochloride (30 mg/ml) every day. At 09.00 h on the day of withdrawal (fifth or tenth day from the start of dosing) the morphine was removed from the reservoirs of one of these groups by washing

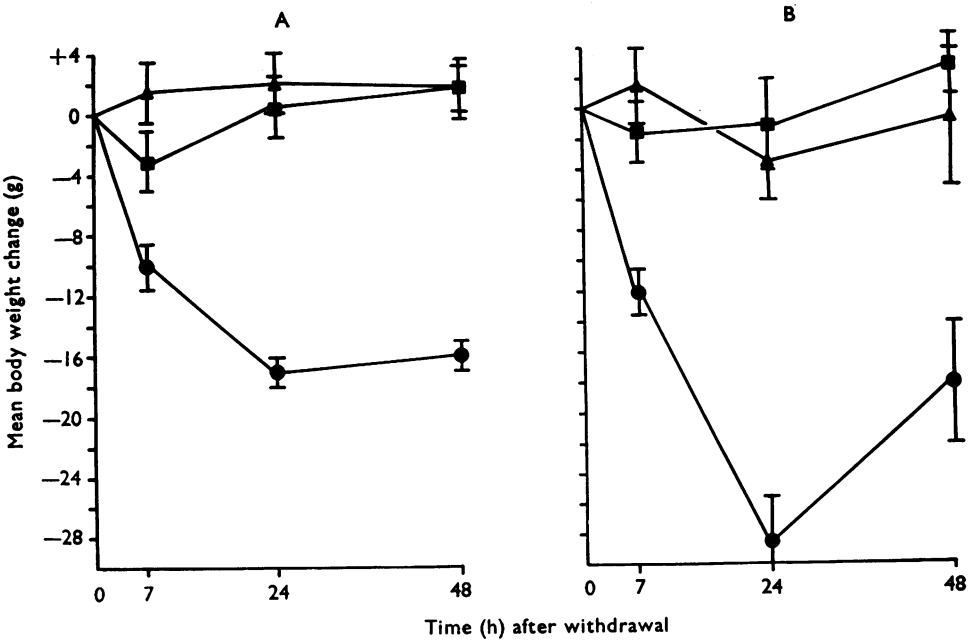


FIG. 3. Effect of morphine withdrawal on rat body weight after 4 days (A) and 9 days (B) of dosing through the reservoir. Morphine withdrawn, ●——●; morphine dosed control (30 mg/ml morphine hydrochloride in reservoir), ▲——▲; vehicle dosed control, ■——■. Rat body weight immediately before withdrawal was in the range 189–274 g. Vertical bars show the standard errors of the means.

through with 6 ml of vehicle. The other group was dosed, as usual, with morphine and acted as a morphine dosed control. Immediately after dosing or withdrawal the rats were placed in individual metabolism cages for 7 hours.

The most striking change following withdrawal of morphine was a rapid loss in body weight which was maximal at 24 h (Fig. 3). Body weight loss was greater on morphine withdrawal after 9 days than after 4 days. Other physiological symptoms

TABLE 1 (a). *Changes in various parameters in rats during morphine withdrawal (tenth day of exposure to drug)*

| Period (withdrawal at 0 h) | Treatment | Mean ± S.E.M. Body weight change (g) | Weight faeces voided (g) | Urine excreted (ml) | Food consumed (g) | Water consumed (ml) |
|---|---|---|---|---|---|---|
| 0–7 h | Vehicle dosed controls ($N=6$) | − 1·5±2·1 | 0·0±0·0 | 2·9±0·6 | 3·0±0·9 | 4·4±1·3 |
| | Morphine dosed controls ($N=5$) | + 1·4±2·5 | 0·2±0·1 | 2·6±0·4 | 4·4±0·5 | 6·1±1·6 |
| | Morphine withdrawn ($N=6$) | −12·2±1·6 † | 3·8±0·8 ‡ | 5·2±0·5 * | 0·8±0·3 * | 2·3±0·9 |
| 0–24 h | Vehicle dosed controls ($N=6$) | − 0·3±3·2 | 6·2±1·5 | 12·7±1·2 | 14·7±2·9 | 24·8±3·1 |
| | Morphine dosed controls ($N=5$) | − 3·6±2·5 | 7·4±0·8 | 14·0±1·7 | 14·0±1·0 | 23·5±1·1 |
| | Morphine withdrawn ($N=6$) | −28·3±3·0 ‡ | 9·1±0·7 | 13·0±1·2 | 4·2±0·9 † | 6·8±1·9 ‡ |

TABLE 1 (b). *Comparison of body weight change and combined net intake of food and water in rats during morphine withdrawal*

| Period (withdrawal at 0 h) | Treatment | Mean values relative to vehicle dosed controls ± S.E.M. Body weight change (g) | Combined net intake of food and water i.e. (weight of food + water)—(weight of faeces + urine) |
|---|---|---|---|
| 0–7 h | Vehicle dosed controls ($N=6$) | 0·0±2·1 | 0·0±2·2 |
| | Morphine dosed controls ($N=5$) | + 2·9±2·5 | + 3·2±1·7 |
| | Morphine withdrawn ($N=6$) | −10·7±1·6 † | −10·4±1·4 † |
| 0–24 h | Vehicle dosed controls ($N=6$) | 0·0±3·2 | 0·0±3·9 |
| | Morphine dosed controls ($N=5$) | − 3·3±2·5 | − 4·5±2·5 |
| | Morphine withdrawn ($N=6$) | −28·0±3·0 ‡ | −31·8±2·9 ‡ |

Significance of difference from vehicle dosed control *$P<0·05$, † $P<0·01$, ‡ $P<0·001$. (Student's $t$ test.)

noted were increased defaecation and urination during the first 7 hours. These increases were significant (defaecation $P<0.01$, urination $P<0.05$) when morphine was withdrawn after 9 days. The faeces were moist and occasionally unformed. The onset of defaecation usually occurred approximately 2 h after morphine had been withdrawn. When the rats, whose morphine was withdrawn after 9 days dosing, were kept in the metabolism cages for a further period (7–24 h after withdrawal), it was found that the weight of faeces voided fell to morphine and vehicle-dosed control levels.

The reason for the body weight loss was examined in the rats in withdrawal after 9 days. It was found (Table 1a) that these rats drank less water ($P<0.001$) during the period 0–24 h after morphine withdrawal and ate less food during both 0–7 h ($P<0.05$) and 0–24 h ($P<0.01$) periods after morphine withdrawal. Furthermore the values for the weight of food and water consumed minus the weight of urine and faeces excreted agreed closely with observed body weight changes in the three groups of rats (Table 1b). It seems likely therefore that body weight loss in these rats in morphine withdrawal was due principally to the effects of hypodipsia and anorexia during the first 24 h exacerbated by increased defaecation during the first 7 hours.

Behavioural symptoms of withdrawal such as abdominal stretching (writhing), 'wet dog' shakes, with squeaking and occasional aggression on handling appeared approximately 1 h after removal of morphine from the reservoir. Writhing and 'wet dog' episodes were most frequent 3 h after withdrawal. Morphine dosed and vehicle dosed control rats showed none of these symptoms.

Both body weight loss and defaecation were the parameters used to study the effects of analgesic compounds on morphine withdrawal symptoms in groups of six rats. In order that there should be no interference from previous treatments each group was used for only one experiment.

TABLE 2. *Effect of analgesics on morphine withdrawal symptoms*

| Drug | Treatment after withdrawal of morphine at 0 h ($N=6$) mg/ml in reservoir | (mg/kg)/day ±S.E.M. | % inhibition of morphine withdrawal symptoms Body weight loss Period 0–7 h | Body weight loss Period 0–24 h | Increased defaecation Period 0–7 h |
|---|---|---|---|---|---|
| Methadone hydrochloride | 30 | 32±1 | 96‡ | 100‡ | 100† |
| Codeine phosphate | 90 | 138±3 | 100‡ | 100‡ | 93* |
| Pethidine hydrochloride | 60 | 110±5 | 54‡ | nil | 92‡ |
| | 90 | 142±3 | 83* | 35 | 91* |
| | 120 | 202±6 | 44 | 1 | 81* |
| Pentazocine hydrochloride | 30 | 19±0·4 | 28 | nil | 20 |
| | 60 | 41±2 | 18 | 48 | nil |
| | 90 | 94±16 | 8 | 34* | 40 |
| Nalorphine hydrobromide | 30 | 42±5 | nil | nil | nil+ |
| Cyclazocine hydrochloride | 5 | 3±1 | nil+ | 49† | nil+ |

Percentage inhibition of morphine withdrawal symptoms $=100\times\frac{(T-W)}{(M-W)}$, where $T$, $W$ and $M$ are mean values for drug substituted, morphine withdrawn and morphine dosed control groups respectively. Significance of difference between morphine withdrawn group and groups which received substitute treatment: * $P<0.05$, † $P<0.01$, ‡ $P<0.001$. (Student's $t$ test.) +Significant ($P<0.05$) increase in withdrawal symptoms.

*Effect of analgesic compounds on morphine withdrawal symptoms*

The effects of substitution of analgesics in the reservoir after 9 days of dosing with morphine are shown in Table 2. Codeine and methadone effectively prevented the morphine withdrawal symptoms of body weight loss and increased defaecation. Pethidine partially substituted for morphine, moderate doses being effective against increased defaecation and body weight loss during the first 7 h, but this drug did not effectively prevent body weight loss which occurred 24 h after withdrawal of morphine from the reservoir.

The opiate-antagonist analgesics pentazocine, nalorphine and cyclazocine caused little or no inhibition of morphine withdrawal symptoms. Nalorphine and cyclazocine in fact caused an increase ($P<0.05$) in defaecation during the first 7 h after morphine withdrawal when compared with morphine withdrawn rats. Cyclazocine in addition, caused an increase ($P<0.05$) in weight loss during the first 7 h followed by an inhibition ($P<0.01$) of weight loss at 24 h, also when compared with morphine withdrawn rats. This indicates an increase in withdrawal symptom intensity followed by a more rapid recovery. Faecal consistency after nalorphine and cyclazocine differed from that of rats in withdrawal alone, the faeces being mostly unformed. In addition there was a more rapid onset of defaecation, the first unformed faeces appearing within 30 min of substituting the drugs for morphine.

*Effects of subcutaneous doses of opiate-antagonists on morphine dependent rats under continued morphine administration*

Morphine dependent rats, having received morphine hydrochloride for 9 days were given a further dose by means of the reservoir and immediately a subcutaneous injection of an opiate-antagonist drug. Naloxone, cyclazocine, nalorphine and pentazocine when administered in this way all precipitated defaecation in non-withdrawn morphine dependent rats (Table 3). In contrast the opiates pethidine, codeine and methadone had no effect on defaecation. Naloxone, cyclazocine and nalorphine also caused a body weight loss in these rats 7 h after injection, but body weight returned to normal 24 h after injection.

TABLE 3. *Effects of subcutaneous doses of opiate-antagonists on morphine dependent rats under continued morphine administration*

| Treatment ($N=6$) | | % precipitation of withdrawal symptoms | | |
|---|---|---|---|---|
| | | Body weight loss | | Weight faeces |
| Drug given at 0 h | Dose mg/kg base (s.c.) | Period 0–7 h | Period 0–24 h | Period 0–7 h |
| Methadone hydrochloride | 10 | nil | 1 | nil |
| Codeine phosphate | 50 | nil | nil | nil |
| Pethidine hydrochloride | 50 | 22 | nil | 21 |
| Pentazocine hydrochloride | 25 | 4 | nil | 30 |
| | 50 | 41 | nil | 31* |
| Nalorphine hydrobromide | 5 | 69† | 1 | 173‡ |
| Cyclazocine hydrochloride | 1 | 85† | nil | 146‡ |
| Naloxone hydrochloride | 1 | 117‡ | nil | 216† |

Percentage precipitation of withdrawal symptoms $=100\times\frac{(M-I)}{(M-W)}$, where $W$, $M$ and $I$ are mean values, for morphine withdrawn, morphine dosed control and (morphine dosed) test drug injected groups respectively. Significance of difference from morphine dosed control: * $P<0.05$, † $P<0.01$, ‡ $P<0.001$. (Student's $t$ test.)

## Discussion

Using the implanted reservoir technique in rats pronounced symptoms were seen on morphine withdrawal after only 9 days of continuous dosing. The morphine withdrawal symptoms were similar to those described by previous authors (Hosoya 1959, Halbach & Eddy, 1963 ; Buckett, 1964). Akera & Brody (1968) considered body weight loss on withdrawal to be the best index of dependence. The results of the preliminary experiments described above, agree with this and, as Madinaveitia (1969) found, the increased weight of faeces voided during the first 7 h also gives an easily measurable and reproducible index of withdrawal.

The method satisfies the criterion of optimal dependence development (Seevers & Deneau, 1963) in that there is continuous ' neuronal exposure ' to morphine and in this respect it resembles the implanted morphine pellet methods of Maggiolo & Huidobro (1961) and Way *et al.* (1969). Another advantage that the reservoir shares with the implanted pellet is that round-the-clock injections of morphine are eliminated. Induction of dependence is achieved with a single daily dose. Dosing by means of the reservoir, however, has the further advantage that the amount of morphine or substitute drug passing from the reservoir can be monitored and controlled at any time. In addition the substitute drug is administered continuously in exactly the same way as morphine.

Rapid production of withdrawal symptoms is obtained simply by washing the morphine from the reservoir with vehicle. Unlike the implanted pellet method withdrawal does not involve any surgery with possible disturbances of the animal and does not differ from the usual daily dosing technique. Also avoided is the delay in the onset of withdrawal symptoms when animals have been made dependent on morphine by normal parenteral injection since these do not occur until the large amount of morphine at the site of the last injection is distributed and metabolized.

Body weight loss and increased defaecation on morphine withdrawal were found to be good indices of the degree of physical dependence, the former being due principally to hypodipsia and anorexia. Both body weight loss and increased defaecation were alleviated partially or completely by doses of the addictive opiate analgesics codeine, methadone and pethidine. In contrast pentazocine, a weak opiate-antagonist analgesic with low addiction liability was ineffective and the antagonist analgesics nalorphine and cyclazocine exacerbated symptoms during the early withdrawal phase. Furthermore the opiate-antagonists pentazocine, nalorphine, cyclazocine and naloxone precipitated symptoms in morphine dependent rats under continued morphine administration, while the opiates codeine, methadone and pethidine had no effect.

It is concluded that this method of dosing with morphine to obtain dependent rats and subsequent examination of the ability of analgesic compounds to prevent withdrawal symptoms will prove useful as a very early assessment of the dependence liability of analgesics.

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